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22469
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit :
Examiner :
Serial No. : 09/975,374
Filed : October 10, 2001
Inventors : Michel Lazdunski
 Gerard Lambeau
 Emmanuel Valentin
Title : CLONING AND
 RECOMBINANT
 EXPRESSION OF
 MAMMALIAN GROUP
 XII SECRETED
 PHOSPHOLIPASE A₂



22469

PATENT TRADEMARK OFFICE

Docket No.: 1479-R-00

Confirmation No.: 1246

Dated: January 4, 2002

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to action on the merits of the case, kindly amend the above-identified application as follows:

In the Specification (Clean copy as amended)

Please replace paragraph 008 (second paragraph on page 3) with the following:

[008] This invention relates to a mammalian secreted group XII sPLA₂ containing a potential Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP.

Please replace paragraph 0010 (last paragraph bridging pages 3 and 4) with the following:

[0010] Fig. 1 represents the alignment of the amino acid sequences of sPLA₂s. In panel A, the full-length sequence of hGXII (SEQ ID NO: 2) is aligned with the amino acid sequences of mouse (SEQ ID NO: 10), rat (SEQ ID NO: 11), bovine (SEQ ID NO: 12) and

Xenopus (SEQ ID NO: 13) GXII sPLA₂s (sequences were deduced from the alignment of different ESTs and from the BAC clone). For some sPLA₂s, the XX residues indicate that the sequence is partial. The *Arrowhead* indicates the predicted signal peptide cleavage site (32). The active site region containing catalytic site residues that are found in all sPLA₂s, and the putative Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP are indicated. The level of identity between the mature protein sequence of hGXII and other GXII sPLA₂s is shown. Panel B shows alignment of the Ca²⁺-binding and active site regions of hGXII (SEQ ID NO: 18) with a representative member of the four other structural classes of sPLA₂s (hGIB (SEQ ID NO: 14) for GI/II/V/X sPLA₂s, hGIII (SEQ ID NO: 15) for GIII sPLA₂s, Conodipine-M (SEQ ID NO: 16) for GIX sPLA₂, and Rice II (SEQ ID NO: 17) for GXI sPLA₂s).

Please replace the paragraph 0015 (last paragraph bridging pages 5 and 6) with the following:

[0015] Thus, the invention concerns a novel mammalian secreted group XII sPLA₂ wherein said enzyme contains a potential Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP. The invention concerns more particularly a mammalian secreted group XII sPLA₂ comprising the sequence of amino acids under SEQ ID NO: 2. More particularly, the mammalian secreted group XII sPLA₂ is a human secreted group XII sPLA₂.

Please replace the 0034 (last paragraph bridging pages 14 and 15) with the following:

[0034] A blastp search with the amino acid sequence of hGXII sPLA₂ against the protein databases stored at the National Center for Biotechnology reveals matches to a variety of sPLA₂s from mammals, *C. elegans*, plants and animal venoms, suggesting that this

protein belongs to the sPLA₂ family. The homology however appears to be weak (< 35% identity with blast scores lower than 35) and restricted to a short stretch of less than 60 amino acid residues containing the active site domain and the HD catalytic diad, indicating that the hGXII sPLA₂ is unique among all known sPLA₂s (Fig. 1B). The histidine of HD is thought to function as a general base to deprotonate a water molecule as it attacks the substrate ester carbonyl carbon, and the β -carboxyl group of the adjacent aspartate coordinates directly to the catalytic Ca²⁺ cofactor (6,33). Except for 3 cysteines in the active site consensus sequence (SEQ ID NO: 9) CCXXHDXC which match those of other groups of sPLA₂s, the location of the other 11 cysteines residues in hGXII is distinct from that of other sPLA₂s (Fig. 1B). Since the structural arrangement of disulfides has been the main basis for designating the different sPLA₂ group numbers, the naming of the new sPLA₂ as hGXII seems appropriate.

Please replace paragraph 0035 (the paragraph bridging pages 15 and 16) with the following:

[0035] The homology between hGXII and all known sPLA₂s is so low that it is difficult to find the Ca²⁺ binding loop, which is usually highly conserved and provides 3 of the 4 amino acid ligands for the catalytic Ca²⁺ (34). All mammalian group I, II, V, and X sPLA₂s contain 19 amino acid residues between the most N-terminal residue that serves as a ligand to the active site Ca²⁺ (i.e. His-27 of hGIIA) and the catalytic histidine (i.e. His-47 of hGIIA). In contrast, the corresponding distances for hGIII and plant GXI sPLA₂s are 25 and 23 residues, respectively, hGXII contains a potential Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP with 23 residues between the N-terminal glycine and the putative catalytic

histidine as shown in Fig. 1. This segment is perfectly conserved among all of the GXII proteins found in gene databases. The x-ray structures of groups I, II, and III sPLA₂s reveal that the Ca²⁺ loop contains the consensus segment X₁CG₁X₂G₂. The backbone carbonyl oxygens of residues X₁, G₁, and G₂ coordinate to Ca²⁺, and the backbone NH of G₁ is proposed to donate a hydrogen bond to the carbonyl oxygen of the enzyme-susceptible substrate ester (33,35). The fact that this residue is glycine in catalytically active sPLA₂s and that mutating this residue to serine lowers catalytic activity by about 10- to 20-fold (35) argues that steric bulk is poorly tolerated at this position. The putative Ca²⁺-coordinating segment of hGXII shown in Fig. 1B fits the consensus sequence of other sPLA₂s with the exception that G₂ is a proline in hGXII. The prediction based on examination of the x-ray structures of sPLA₂s is that the hGXII Ca²⁺ binding segment should be functional. It contains G₁, and the backbone carbonyl of the C-terminal proline can coordinate to Ca²⁺ since its three extra methylenes, compared to glycine, are sterically allowed because of the location of this residue on the enzyme's surface away from the substrate binding cavity. Interestingly, sPLA₂ isozymes with relatively low sPLA₂ activity from the venom of the banded krait also contain proline in place of G₂ (36).

In the Claims (Clean copy as amended)

1) (Amended) A mammalian secreted group XII sPLA₂ containing a potential Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP.

In the Abstract (Clean copy as amended)

This invention concerns DNA and peptide sequences encoding a novel mammalian secreted group XII phospholipase A₂ wherein said enzyme contains a potential Ca²⁺ binding segment (SEQ ID NO: 8) GCGSP and more particularly, a novel human group XII phospholipase A₂. The invention also concerns the use of this enzyme in methods for screening various chemical compounds.

Remarks

We respectfully request that the above-identified amendments entered into the file of the case. They are made to insert sequence ID numbers into the Specification and no new matter has been added.

An early action on the merits of the case is respectfully requested.

Respectfully submitted,



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